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NEUTRAL LIPIDS OF *Ruta graveolens* L. GROWN *in vivo* AND *in vitro*

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The composition of neutral lipids (NL) and fatty acids of acyl-containing components in differentiated tissues of Ruta graveolens *L. (Rutaceae) grown in vivo and in vitro is studied. The NL of in vitro cultures differ from NL of the intact plant by a larger content of esters of fatty acids with alcohols, fewer high-molecular-weight alkanes, alkylbenzenes, free alkanols, and linolenic acid.*

Key words: *Ruta graveolens*, *in vitro*, neutral lipids, fatty acids.

It was reported earlier [1, 2] that a culture of *Ruta graveolens* L. (Rutaceae) that is grown by callucogenesis and regeneration (*in vitro*) retains the ability to biosynthesize the principal lipid components and secondary metabolites that are characteristic of the native plant (*in vivo*). The content of neutral lipids (NL) increases in the *in vitro* culture biomass compared with the *in vivo* biomass.

In continuation of the study of lipids from this plant, NL were isolated from the *in vivo* (I) and *in vitro* (II) biomass and separated into pure fractions using column chromatography on silica gel. The content of NL components calculated in mg/g dry wt. and % of NL mass is listed in Table 1.

This research indicates that the content of both acylated and unacylated NL components changes substantially with increasing NL level in the tissues of culture II compared with culture I.

Examination of the NL components in mg/g dry wt (Table 1) shows that hydrocarbons dominate in tissues of the intact plant. However, tissues of culture II contain substantially more free fatty acids (FFA), ester of fatty acids with alcohols (FAEA), high-molecular-weight aliphatic alcohols, phytosterols (1.5-2.4 times), and unidentified substances (3.2 times) than culture I. The triacylglycerine (TAG) content in both samples is almost identical.

The NL components (% of mass) of cultures I and II differ significantly by a sharp decrease in the hydrocarbon fraction (by 2.9 times). The total content of acylated lipids is greater in tissues of culture II (37.75%) than in I (32.1%). This is due mainly to FAEA. The content of FFA in the NL is almost equal in the two samples.

TLC and UV and mass spectra [3] of hydrocarbons in cultures I and II demonstrated that they consist of alkanes, alkenes, alkadienes, alkatrienes, and alkylbenzenes. The alkenes, alkadienes, and alkatrienes are present in the hydrocarbons of I and II in small quantities as homologs with C_{19}-C_{35} chain lengths. The mass numbers and intensities of the principal alkane and alkylbenzene molecular ions in I and II are listed in Table 2.

The alkanes and alkylbenzenes of both samples consist of $C_{19\text{-}35}H_{40\text{-}72}$ and $C_{19\text{-}35}H_{32\text{-}64}$ homologs, respectively. Uneven homologs with predominantly gentriacontane $C_{31}H_{64}$ dominate the hydrocarbons of the intact plant. Peaks of the M^+ ions, which belong to alkanes with a chain length from C_{19} to C_{25} and alkylbenzenes with chain length from C_{19} to C_{29}, are stronger in mass spectra of hydrocarbons from the *in vitro* culture.

Therefore, growing *R. graveolens* under *in vitro* conditions decreases the hydrocarbon level in tissues. The decreased content of high-molecular-weight homologs of alkanes and alkylbenzenes that are characteristic of the intact plant is more noticeable.

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TABLE 1. Composition of Neutral Lipids of *R. graveolens* Culture I and II

Component	Composition of neutral lipids			
	mg/g dry wt		% of mass	
	I	II	I	II
Hydrocarbons	7.36	4.44	18.41	7.30
α- and β-Carotenes	0.04	0.01	0.11	0.02
Fatty-acid esters with aliphatic alcohols and β-sitosterol	4.36	9.66	10.90	15.89
Methyl and butyl fatty-acid esters	1.62	2.27	4.04	3.73
Yellow pigment	0.06	0.01	0.16	0.02
Triacylglycerines	3.55	4.00	8.86	6.58
Orange and red pigments	0.01	0.01	0.02	0.02
Unidentified	4.28	9.53	10.70	15.67
Free fatty acids	3.32	5.20	8.30	8.55
High-molecular-weight alcohols and triterpenes	4.96	9.11	12.41	14.98
4-Monomethylsterols, 4,4-dimethylsterols, and xanthophylls	3.87	5.67	9.67	9.33
Native and altered chlorophylls together with an unidentified substance	6.57	10.89	16.42	17.91
Total NL	40.00	60.80	100	100

TABLE 2. Composition of Alkanes and Alkylbenzenes of *R. graveolens* Culture I and II According to Mass Spectra

Number of C atoms	C_nH_{2n+2} alkanes			C_nH_{2n-6}*alkylbenzenes		
	M^+, *m/z*	Relative intensity, %		M^+, *m/z*	Relative intensity, %	
		I	II		I	II
C_{35}	492	0.1	0.1	484	0.1	0.1
C_{34}	478	0.1	0.1	470	0.1	0.1
C_{33}	464	4.2	0.1	456	0.2	0.2
C_{32}	450	0.6	0.2	442	0.4	0.3
C_{31}	436	11.0	0.4	428	0.8	0.6
C_{30}	422	0.9	0.5	414	1.1	1.6
C_{29}	408	2.7	0.5	400	1.4	2.7
C_{28}	394	2.0	1.0	386	1.5	3.1
C_{27}	380	4.2	1.3	372	1.6	4.0
C_{26}	366	3.0	1.8	358	1.0	3.0
C_{25}	352	3.6	2.3	344	1.0	3.3
C_{24}	338	3.3	2.9	330	1.0	4.3
C_{23}	324	2.7	3.0	316	0.9	4.5
C_{22}	310	2.4	3.2	302	0.7	4.8
C_{21}	296	2.0	3.6	288	0.8	5.2
C_{20}	382	2.0	3.4	274	0.6	4.1
C_{19}	268	2.1	3.5	260	1.0	4.5

*Diagnostic ions for alkylbenzenes I and II with *m/z* 148 (1.4 and 1.6), 147 (2.0 and 8.7), 134 (1.8 and 7.6), 133 (3.2 and 13.0), 119 (4.1 and 16.8), 105 (4.1 and 17.6), 91 (3.7 and 15.2), 57 (100 and 100), and 43 (92 and 93, respectively).

The component composition of FAEA in I and II was studied by TLC and mass spectra [3] taking into account the fatty-acid composition (Table 3) and was identical. The main components of the FAEA are waxy esters of aliphatic alcohols $C_{20-32}H_{41-65}OH$. According to mass spectra, the waxy esters are a mixture of homologs from $C_{34}H_{68}O_2$ to $C_{48}H_{96}O_2$ (M^+ 508-704) with the even components dominating. The ester of β-sitosterol with linoleic acid was identified by the presence in the mass spectrum of peaks for the molecular ion M^+ 676 and fragments with *m/z* 535, 397, 396, 296, and 255 [4]. Furthermore, peaks for molecular ions M^+ 534, 562, and 560 and diagnostic fragments with *m/z* 278, 125, 124, 111, 97, 85, 83, 71, and 69

TABLE 3. Fatty-acid Composition of NL in *R. graveolens* Culture I and II, % by GLC

Acid	Triacylglycerines		FAEA		FAE		FFA	
	I	II	I	II	I	II	I	II
12:0	0.7	1.0	1.7	2.5	-	-	0.3	1.3
14:0	2.5	2.7	3.0	10.7	4.3	Tr.	1.8	4.5
15:0	0.4	0.5	0.7	2.0	1.1	Tr.	Tr.	1.5
16:0	15.7	17.1	28.4	32.1	58.6	45.7	26.6	33.8
16:1	3.5	2.7	Tr.	Tr.	-	-	0.9	0.2
17:0	Tr.	Tr.	1.6	1.9	Tr.	Tr.	Tr.	Tr.
18:0	3.0	4.0	35.5	26.4	16.1	7.6	4.5	9.4
18:1	16.1	15.9	14.2	13.0	9.0	15.9	10.9	10.7
18:2	38.2	38.0	10.5	2.6	4.9	23.4	16.2	18.8
18:3	19.9	18.1	2.7	2.5	6.0	7.4	38.8	8.8
20:0	Tr.	Tr.	1.7	6.3	Tr.	Tr.	Tr.	2.0
22:0 -26:0	-	-	-	-	-	-	Tr.	Tr.
$\sum_{sat}$	22.3	25.3	72.6	81.9	80.1	53.3	33.2	52.5
$\sum_{unsat}$	77.7	74.7	27.4	18.1	19.9	46.7	66.8	47.5

were observed in the mass spectrum [5]. These belong to phytyl esters of 16:0, 18:0, and 18:1 acids.

Lipids of I and II yielded methyl and butyl esters of fatty acids (FAE). These were identified using GLC and mass spectrometry [6]. Tissues of culture II contain more of them than those of I.

The mass spectrum of FAE exhibited characteristic peaks for the methyl esters of fatty acids. The butyl ester of 16:0 acid was identified in the FAE fraction by comparing the mass spectrum (M^+ 312) and GLC (equivalent chain length 16.69) with the literature [7]. The presence in the mass spectrum of M^+ 340 suggests the presence of the butyl ester of 18:0 acid.

Thus, rarely encountered methyl and butyl esters of fatty acids are present in photosynthetic tissues of cultures I and II of *R. graveolens.* Of these, the butyl ester of 16:0 is the second time this lipid component has been observed in plants of the Rutaceae family [7]. The methyl esters are identified for the first time. Natural methyl esters of fatty acids have been observed in ripe [8] and ripening seeds [6], surface waxes of higher plants [9], algae [10], and photosynthetic bacteria [11]. However, their physiological role in plant tissues is still unclear. It was proposed [9] that they may act as promoters of tissue growth stimulators.

The fatty-acid composition of TAG and FAE was determined by mild alkaline hydrolysis; of FAEA, strong. Acids isolated from the hydrolysis products and FFA were analyzed by GLC of the methyl esters. Their composition is given in Table 3.

Table 3 shows that the TAG of cultures I and II differ little in fatty-acid content. The overall content of unsaturated acids reaches >70% of the total mass. The dominant acid is 18:2. On the other hand, FAE and FAEA in the studied samples are enriched with saturated acids: FAE, 16:0; FAEA, both 16:0 and 18:0. The composition of FFA and FAEA of culture II exhibits a tendency to increase the fraction of saturated acids compared with that of culture I whereas that of the FAE has increased unsaturated acids 18:1 and 18:2. The sharp decrease of 18:3 acid in FFA of culture II compared with culture I is notable. According to the literature [12], 18:3 acid occurs mainly in polar-lipid acyls that organize membranes of photosynthetic tissues by supporting their structural integrity and functional activity. We previously observed in *in vitro R. graveolens* culture a decrease in the content of 18:3 acid esterified at all individual components of glyco- and phospholipids, especially in phosphatidylcholine.

Thus, the results suggest that the photosynthetic activity of chloroplasts and the formation in *R. graveolens* tissues of free linolenic acid and *sn*-linolenoyl-containing polar lipids are directly related [2].

EXPERIMENTAL

General comments have been published [1].

The esters of β-sitosterol with linoleic acid, waxy esters, and methyl and butyl esters were identified by their

chromatographic mobility on TLC (silica gel); their saponification to form the corresponding fatty alcohols, β-sitosterol, and fatty acids; and the GLC behavior of fatty-acid methyl and butyl esters. UV, IR, and mass spectra and the GLC and TLC behavior of pure NL and fatty-acid classes correspond to those in the literature [1, 3].

REFERENCES

1. D. T. Asilbekova, S. D. Gusakova, A. I. Glushenkova, A. R. Azizkhodzhaev, E. M. Erkkenova, and M. Sakhibaeva, *Khim. Prir. Soedin.*, 651 (1993).
2. D. T. Asilbekova, S. D. Gusakova, A. I. Glushenkova, and I. A. Bessonova, *Khim. Prir. Soedin.*, 215 (1995).
3. D. T. Asilbekova, S. D. Gusakova, Yu. M. Murdakhaev, and A. I. Glushenkova, *Khim. Prir. Soedin.*, 615 (1994).
4. Ya. I. Rashkes, N. K. Khidyrova, A. M. Rashkes, and Kh. M. Shakhidoyatov, *Khim. Prir. Soedin.*, 208 (1990).
5. P. A. Cranwell, G. H. Jaworski, and H. M. Bickley, *Phytochemistry*, **29**, 145 (1990).
6. S. G. Yunusova and S. D. Gusakova, *Khim. Prir. Soedin.*, **40**, 44 (1982).
7. J. S. Shah and B. K. Sabata, *J. Indian Chem. Soc.*, **58**, No. 11, 1123 (1981).
8. T. V. Panekina, S. D. Gusakova, and A. U. Umarov, *Khim. Prir. Soedin.*, 174 (1978).
9. M. I. Chu, M. A. Wheeler, and C. E. Holmlund, *Biochim. Biophys. Acta*, **270**, 18 (1972).
10. T. V. Khomova, S. D. Gusakova, and A. I. Glushenkova, *Khim. Prir. Soedin.*, 325 (1994).
11. B. M. Maudinar and J. Villoutreix, *Phytochemistry*, **16**, 1299 (1977).
12. M. Kates and V. O. Marshall, in: *Recent Advances in the Chemistry and Biochemistry of Plant Lipids*, T. Galliard and F. Mercer, eds., Academic Press, London-New York-San Francisco (1975), p. 225.